American Journal of Pathology, Vol. 168, No. 1, January 2006

DOI: 10.2353/ajpath.2006.041089

Epithelial and Mesenchymal Cell Biology

Matrix Metalloproteinase Inhibitors Suppress Transforming Growth Factor-β-Induced Subcapsular Cataract Formation

Dhruva J. Dwivedi,* Giuseppe Pino,* Alice Banh,† Zahra Nathu,* Derek Howchin,* Peter Margetts,‡ Jacob G. Sivak,† and Judith A. West-Mays*

From the Department of Pathology and Molecular Medicine and the Division of Nephrology,‡ McMaster University, Hamilton; and the School of Optometry,† University of Waterloo, Waterloo, Ontario, Canada*

The pleotropic morphogen transforming growth factor-β (TGFβ) plays an important role in the development of fibrotic pathologies, including anterior subcapsular cataracts (ASCs). ASC formation involves increased proliferation and transition of lens epithelial cells into myofibroblasts, through epithelial-mesenchymal transformation that results in opaque plaques beneath the lens capsule. In this study, we used a previously established TGFβ-induced rat cataract model to explore the role of matrix metalloproteinases (MMPs) in ASC formation. Treatment of excised rat lenses with TGFβ resulted in enhanced secretion of MMP-2 and MMP-9. Importantly, co-treatment with two different MMP inhibitors (MMPIs), the broad spectrum inhibitor GM6001 and an MMP-2/9-specific inhibitor, suppressed TGFβ-induced ASC changes, including the epithelial-mesenchymal transformation of lens epithelial cells. Using an anti-E-cadherin antibody, we revealed that conditioned media from lenses treated with TGFβ contained a 72-kd E-cadherin fragment, indicative of E-cadherin shedding. This was accompanied by attenuated levels of *E-cadherin* mRNA. Conditioned media from lenses co-treated with TGFβ and MMPIs exhibited attenuated levels of the E-cadherin fragment compared with those from TGFβ-treated lenses. Together, these findings demonstrate that TGFβ-induced E-cadherin shedding in the lens is mediated by MMPs and that suppression of this phenomenon might explain the mechanism by which MMPIs inhibit ASC plaque formation. ***(Am J Pathol 2006, 168:69–79; DOI: 10.2353/ajpath.2006.041089)***

Loss of transparency of the lens, or cataract, is the leading cause of blindness worldwide, despite the availability of effective surgery in developed countries.[1] Currently, there are no pharmacological agents to prevent the onset or to inhibit the progression of cataract formation. Thus, an understanding of the cellular and molecular mechanisms regulating the normal and pathological differentiation of the lens may lead to the development of therapeutic strategies for the treatment and/or prevention of cataracts.

The lens is a relatively simple tissue composed of two cell types: epithelial cells and fiber cells. In the embryo, the lens consists of a highly proliferative monolayer of lens epithelial cells (LECs) that cover the anterior half of the lens.[2] At the lens equator, these cells are stimulated to terminally differentiate into fiber cells by a gradient concentration of growth factors within the ocular media.[3] In adults, lens proliferation and differentiation occurs near the lens equator, albeit at a slower rate than in the embryo. However, in a pathological situation such as occurs after ocular trauma, surgery, or systemic diseases like atopic dermatitis and retinitis pigmentosa, the anterior LECs can be triggered to proliferate and multilayer beneath the lens capsule.[3,4] A proportion of these cells transform into plaques of large "spindle shaped" cells, or myofibroblasts, by a phenomenon known as epithelial-to-mesenchymal transition (EMT).[5–7]

The cytokine transforming growth factor-β (TGFβ) has been shown to play a role in lens disease and to induce these aberrant changes in LECs, including their conversion to myofibroblasts.[3] The resultant myofibroblasts express contractile elements, like α-smooth muscle actin

Supported by research grants from the National Institutes of Health project grants (EY013845-01 and EY015006-01 to J.A.W.-M.), Canada Foundation for Innovation (to J.A.W.-M.), and Natural Sciences and Engineering Research Council of Canada (to J.G.S.). J.A.W.-M. was a Research to Prevent Blindness Career Development Awardee (2000–2003).

D.J.D. and G.P. contributed equally to this study.

Accepted for publication August 30, 2005.

Address reprint requests to Judith A. West-Mays, Ph.D., Department of Pathology and Molecular Medicine, McMaster University, HSC 1R10, Hamilton, ON, Canada L8N3Z5. E-mail: westmayj@mcmaster.ca.

(α-SMA), and begin to secrete an abnormal accumulation of type I and III collagen.[8] Additional extracellular matrix proteins are deposited, including tenascin and fibronectin, and as a result, fibrous anterior subcapsular cataract (ASC) plaques form that develop into distinct opacities in the lens. Similar to ASC, in secondary cataract, also known as posterior capsular opacification, LECs that remain within the capsule after cataract surgery are triggered to proliferate and migrate to the posterior lens capsule, where they frequently transform into myofibroblasts.[9,10]

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that act as key regulators of tissue remodeling[11] and have been shown to participate in a number of ocular diseases, including retinal disease, glaucoma, and corneal disorders.[12] MMPs and tissue inhibitors of matrix metalloproteinases have also been examined in the normal and cataractous lens. Although some MMPs, such as MMP-9 and membrane-type 1-MMP, have been found to be constitutively expressed in the lens,[13–15] other MMPs, such as MMP-2, are typically expressed after treatment with growth factors or during cataract formation. For example, Seomun et al[16] reported that TGFβ triggers MMP-2 mRNA expression in a human lens epithelial cell line and induces immunoreactivity for both MMP-2 and α-SMA in the subcapsular plaques of rat lenses. TGFβ has also been shown to stimulate secretion of MMP-2 and MMP-9 in cultured annular pad cells of the chick lens[17] and in human capsular bags.[18] Induction of the proforms of both MMP-2 and MMP-9 has also been reported during hydrogen peroxide-induced cataract formation and after sham cataract surgery.[19] Multiple MMPs and tissue inhibitors of matrix metalloproteinases have also been detected in the extracellular matrix and LECs from human capsules derived from post-cataract/intraocular lens surgery tissue, whereas normal anterior lens capsules did not.[20]

Evidence that MMP-2 may have an active role in mediating the EMT that occurs in ASC has been provided by findings in which overexpression of MMP-2 in the human HLE B-3 lens cell line caused a conversion of the cells into a myofibroblastic phenotype.[16] The ability of other MMPs to induce LEC conversion, however, has not been investigated. In addition, the requirement for MMPs in mediating the formation of the TGFβ-induced ASC plaques in the *ex vivo* lens and the mechanism(s) by which MMPs participate in ASC formation are not known. Investigations in other systems, including cancer, have shown that MMPs promote EMT by altering the E-cadherin/β-catenin pathway through a phenomenon known as E-cadherin shedding.[21–24] Specifically, the association between E-cadherin and β-catenin is vulnerable to enzymatic attack by multiple MMPs, including MMP-9 and MMP-2, resulting in the formation of E-cadherin extracellular domain fragments with reported sizes ranging from 50 to 84 kd.[24–26] Induction of MMPs by TGFβ in the lens may lead to the EMT of LECs through a specific disruption in E-cadherin.

In the current study we directly tested the requirement of MMP activity in TGFβ-induced ASC formation using a well-established *ex vivo* rat lens model[27] in conjunction with the two different MMP inhibitors (MMPIs), the broad spectrum inhibitor GM6001 and a MMP-2/9-specific inhibitor (MMPI2/9). Additional experiments examined the capacity of these MMPIs to prevent decreases in optical quality of the lens induced by TGFβ and to affect the cell adhesion molecule E-cadherin. Together, the findings of this study demonstrate that co-culture of lenses with two different MMPIs effectively suppressed TGFβ-induced ASC formation and suggest attenuation of E-cadherin as a possible mechanism.

Materials and Methods

Ex Vivo *Rat Lens Cataract Model*

The previously established TGFβ-induced rat cataract model was used for these studies.[27] Briefly, lenses were obtained from adult male Wistar rats and cultured overnight in 3.5 ml of serum-free M199 medium supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin, and 2.5 μg/ml fungizone (Invitrogen, Burlington, ON, Canada). The following day, lenses were either left untreated or treated with TGFβ-2 (R&D Systems, Minneapolis, MN) at a final concentration of 1 or 2 ng/ml. Some lenses were co-treated with TGFβ-2 and the broad spectrum MMP inhibitor GM6001 (Ilomastat) (Chemicon International, Temecula, CA) at concentrations ranging from 10 to 25 μmol/L or the MMP-2/9 inhibitor ((2*R*)-[(4-biphenylylsulfonyl)amino]-*N*-hydroxyl-3-phenylpropionamide) (Chemicon) at concentrations of 10 or 25 μmol/L. The GM6001 negative control (*N*-*t*-butoxycarbonyl-L-leucyl-L-tryptophan methylamide) (25 μmol/L) (Calbiochem, San Diego, CA) was also used. Lenses were subjected to optical analysis at subsequent time points of 2, 4, or 6 days and photographed using a digital camera mounted to a dissecting scope. The lenses were then fixed for histology and immunofluorescence or subjected to laser capture microdissection (LCM). The conditioned media were also collected from each treatment group for zymography and Western blot analysis.

Optical Analysis

Lens optical qualities (the average back vertex distance [BVD]) and sharpness of focus (BVD error) were assessed using the automated laser scanning system that was developed at the University of Waterloo.[28–30] This system consists of a single collimated scanning helium-neon laser source that projects a thin (0.05 mm) laser beam onto a plain mirror mounted at 45° on a carriage assembly. A digital camera captures the actual position and slope of the laser beam at each step. When all steps have been made (20 beam positions across each lens), the captured data for each step position are used to calculate the back vertex distance. BVD (in millimeters) is defined as the measurement of the distance from the surface of the lens to the focal point where the laser beam crosses the optical axis of the lens being scanned. When portrayed graphically, the average BVD for the lenses is

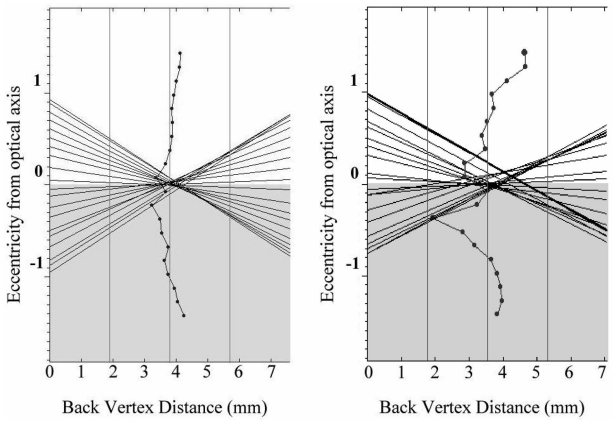


Figure 1. Optical scans of rat lenses. The scatter plots represent the back vertex measurements (focal length) of a control lens (**left**) and a TGFβ-treated lens (**right**). The *y* axis indicates the eccentricity of the laser beam from the optical axis and the *x* axis demonstrates BVD measurements. Each point on the scatter plot represents the back vertex distance from each beam location. In a control (noncataractous) lens, there is little difference in back vertex distance, demonstrating sharpness in focus. In a TGFβ-treated lens, there is substantial variation in the back vertex distances.

plotted for each eccentric position (Figure 1). With less spherical aberration, the data points line up as a straight line. The poorer the quality of the lens, the greater the variation in BVD (BVD error) is with eccentricity, as shown for lenses treated with TGFβ (Figure 1). Because BVD error is a more sensitive measure of lens damage, the results are expressed in terms of BVD error.[28–30] Repeated-measures analysis of variance was determined using SPSS 11.0 statistical software and was used to assess treatment, concentration of TGFβ, and temporal effects on the back vertex variability for the first experiment in Figure 2. One-way analysis of variance was used for analyzing data in the second experiment in Figure 3, which examines the dosage effect of GM6001 on TGFβ-induced BVD error at a single time point. A probability value (P) ≤0.05, indicating a 95% confidence interval, was considered significant.

Histology and Immunohistochemistry

Lenses were collected from different treatment groups and fixed overnight in an acetic acid:ethanol solution (1:99), dehydrated, embedded in paraffin, and processed for routine histology. Immunofluorescence was performed on 5-μm-thick paraffin-embedded sections. Sections were incubated with primary antibody specific for α-SMA (1:100; Sigma, Oakville, ON, Canada) for 1 hour at room temperature, and bound primary antibodies were visualized with a fluorescein-isothiocyanate anti-mouse secondary antibody (1:50; Jackson ImmunoResearch Laboratories, West Grove, PA). All sections were mounted in Vectashield mounting medium with 4′,6-diaminodino-2-phenylindol (Vector Laboratories, Burlington, ON, Canada) to visualize the nuclei.

Zymography

Conditioned media from all treatment groups were concentrated using 3.5-ml 10K Microsep concentrating devices (Viva Sciences, Hanover, Germany). Before concentration, refrigerated media were warmed to 37°C. The media were centrifuged at 1000 × *g* (at room temperature) for 5 minutes to pellet any debris before loading. Each device was loaded with an equal volume of supernatant, and the concentration was performed by centrifugation at 25°C for 30 minutes at 3000 × *g*. An equal

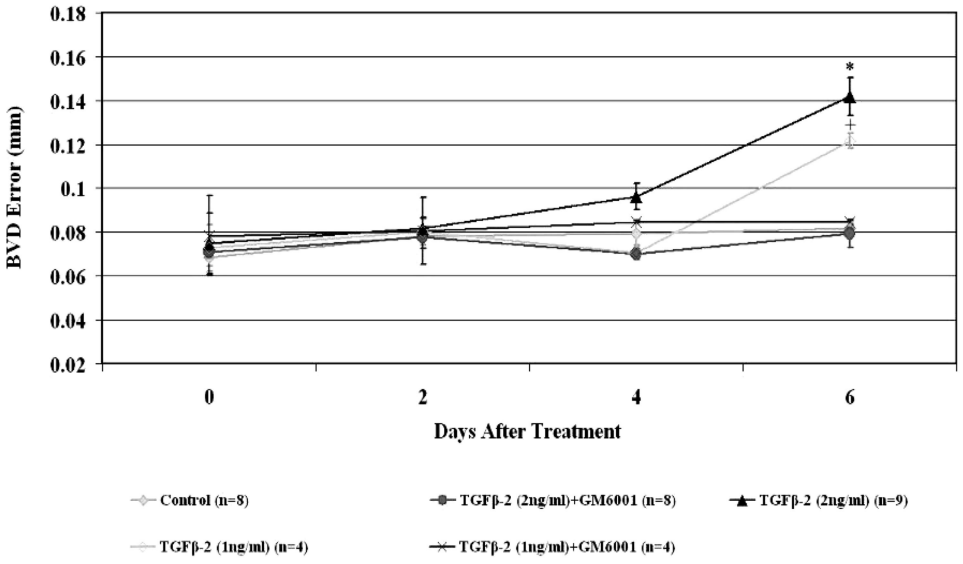


Figure 2. Back vertex variability for lenses left untreated, treated with TGFβ, and treated with TGFβ and GM6001. The graph shows the changes in back vertex variability (BVD error; mm ± SEM) from day 0 (initial measurements before treatment) and 2, 4, and 6 days after treatment. Two concentrations of TGFβ (1 and 2 ng/ml) were used in this experiment. Repeated-measures analysis of variance demonstrated that there are both treatment and temporal effects ($P \leq 0.05$). At day 6 after treatment, both concentrations of TGFβ ($^{+}$1 and *2 ng/ml) showed significantly higher BVD error measurements than other treatment groups, including the control and the TGFβ (1 ng/ml), GM6001 and TGFβ (2 ng/ml), and GM6001 groups. Apparent differences between the TGFβ (1 ng/ml) and TGFβ (2 ng/ml) groups did not reach statistical significance.

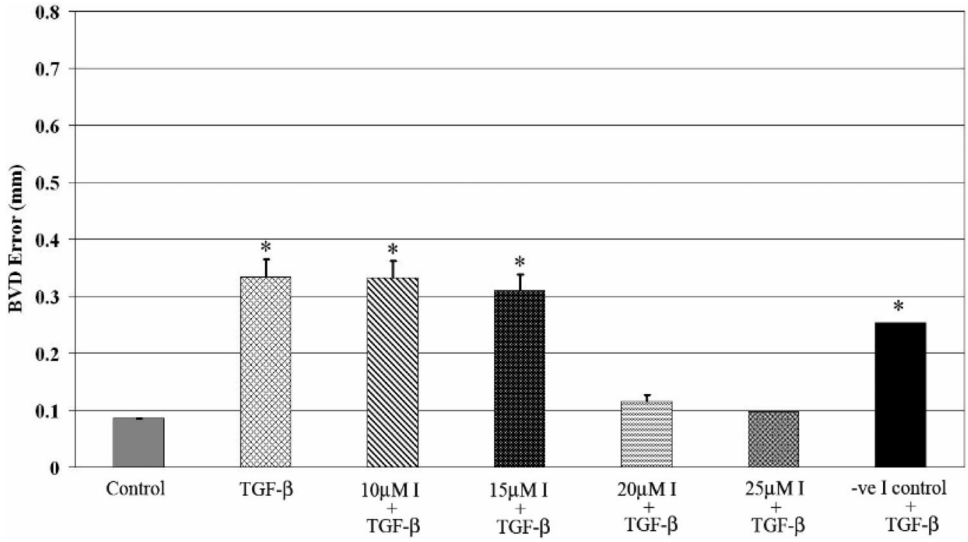


Figure 3. Dosage effect of GM6001 on TGFβ-induced BVD variability. This bar graph represents the back vertex variability (BVD error, mm) ± SEM of lenses left untreated or treated with TGFβ (2 ng/ml) or TGFβ (2 ng/ml) plus four different concentrations of GM6001 (I) as indicated for 6 days. These measurements show a decrease in BVD error as the GM6001 concentration increases to 25 μmol/L. One-way analysis of variance was used to determine the dosage effect of GM6001 on TGFβ-treated lenses ($P \leq 0.05$). BVD errors from TGFβ-treated lenses co-treated with 20 or 25 μmol/L GM6001 were not significantly different from the control lenses. In contrast, lenses treated with TGFβ (2 ng/ml) alone or with TGFβ (2 ng/ml) plus GM6001 at 10 and 15 μmol/L had BVD errors that were significantly different from control lenses, as indicated by **asterisks**. Lenses treated with TGFβ and the negative control for GM6001 (25 μmol/L) also had BVD errors that were significantly different from the controls.

volume of each concentrate was electrophoresed on 10% SDS-polyacrylamide gels containing 0.1% gelatin as the substrate. After electrophoresis, the gels were developed as described previously[31] and stained in 0.5% Coomassie brilliant blue for 1 hour followed by destaining with 10% isopropanol. Sites of gelatinase activity were detected as clear bands against a background of uniform staining, which was digitally photographed.

Western Blot Analysis

Concentrated samples derived from the conditioned media were also examined by Western blot analysis. Equal volumes of sample were electrophoresed on a 10% SDS-polyacrylamide gel. The resolved bands were electrotransferred onto a nitrocellulose membrane (Pall Corporation, Pensacola, FL). Membranes were blocked with 5% skimmed milk powder in Tris-buffered saline (50 mmol/L Tris base and NaCl [pH 8.5]) and 0.1% Tween-20 and then incubated overnight at 4°C with primary antibodies generated against MMP-9 (1:500; Chemicon International), MMP-2 (1:500; Chemicon), or E-cadherin (1:1500; BD Transduction Laboratories). After this incubation, membranes were probed with an horseradish peroxidase-conjugated secondary antibody (1:7500; Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and ECL detection reagents (Amersham Biosciences). The Western blots were scanned and analyzed by image quantification software (ImageJ; National Institutes of Health, Bethesda, MD). The relative density versus control ratio was estimated using GraphPad Prism Software (GraphPad). Quantitative data were analyzed statistically using a Student's *t*-test and expressed as mean ± SEM. A value of $P < 0.05$ was considered significant.

Laser Capture Microdissection, RNA Extraction, and cDNA Synthesis

After treatment, lenses were placed in a cryostat mold containing Tissue-Tek OCT (Sakura Finetek, Torrance, CA), frozen on dry ice, and then stored at −70°C. The frozen tissue was then sectioned at 5 to 8 μm in a cryostat, mounted on noncoated clean glass slides, and stored again at −70°C. Immediately before LCM, the frozen sections were thawed for 10 seconds and then stained with HistoGene LCM Frozen Section Staining kit (Arcturus, Mountain View, CA) using the protocol provided, with strict adherence to RNase-free conditions. LCM was then performed using the PixCell II (Arcturus), as described by others.[32,33] The HistoGene stain allowed for the identification of the general morphology of the epithelium. Cells from the epithelial region of the lens were then captured on CapSure Macro LCM Caps (Arcturus) using the PixCell II LCM Microscope (Arcturus) with a minimal beam diameter of 7.5 μm. Total cellular RNA was then extracted from lifted cells using the PicoPure RNA Isolation kit (Arcturus). Purified RNA was analyzed both qualitatively and quantitatively using an Agilent 2100 Bioanalyzer (Agilent Technologies, Foster City, CA) and subjected to standard reverse transcription reactions (SuperScript II; Life Technologies).

Quantification of Gene Expression Using Real Time Quantitative Polymerase Chain Reaction (RT-QPCR)

E-cadherin and *α-SMA* gene expression from recovered cDNA was analyzed with RT-PCR using a 96-well

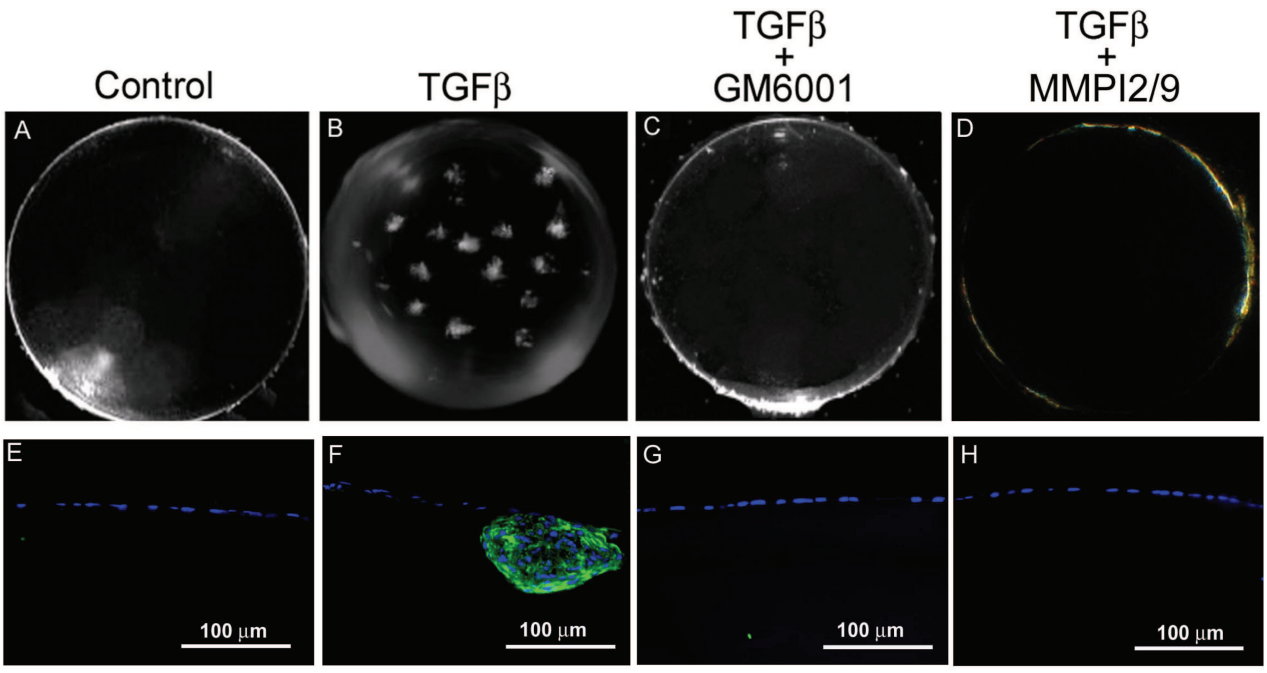


Figure 4. TGFβ-induced subcapsular formation in the rat lens is inhibited by co-culture with GM6001 and specific MMP-2/9 inhibitor. An untreated control lens (**A**), a lens treated with TGFβ (2 ng/ml) (**B**), a lens co-cultured with TGFβ (2 ng/ml) and GM6001 (25 μmol/L) (**C**), and a lens co-cultured with TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (**D**) are shown after 6 days of culture. The TGFβ (2 ng/ml)-treated lens (**B**) exhibited distinct subcapsular plaques unlike the untreated lens (**A**) or those co-cultured with GM6001 (25 μmol/L) (**C**) and MMPI2/9 (25 μmol/L) (**D**), which remained devoid of opacities. Immunolocalization of α-SMA in cross-sections of lenses revealed strong immunoreactivity of α-SMA (green) in sections of lenses treated with TGFβ (2 ng/ml) (**F**), confirming the presence of subcapsular plaques. Control lenses (**E**), lenses co-cultured with TGFβ (2 ng/ml) and GM6001 (25 μmol/L) (**G**), and lenses co-cultured with TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (**H**) showed no observable immunoreactivity to α-SMA. All sections were mounted in a medium with 4′,6-diaminodino-2-phenylindol to co-localize the nuclei (blue). Scale bars = 100 μm.

TaqMan optical reaction plate format on an ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). RNA was normalized to *18S* and *GAPDH* for each reaction. Each 25-μl PCR reaction (including controls) contained TaqMan Universal Master Mix (Applied Biosystems), gene-specific forward and reverse primers (Mobix, Hamilton, ON, Canada), and probes for target and endogenous control genes (Applied Biosystems). Serial dilutions (one- to fivefold) of standard samples were prepared in separate wells in duplicate for endogenous control genes (*18S* and *GAPDH*), *E-cadherin*, and *α-SMA* gene targets. Standard and unknown samples were added in a volume of 5 μl. Thermal cycling parameters consisted of the following: 2 minutes at 50°C, 10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C, and 1 minute at 60°C. The number of target gene copies was calculated from a standard curve generated in parallel with each batch of samples. A linear relationship was detected over at least 5 orders of magnitude. In all experiments, the correlation coefficient was between 0.997 and 0.986. The normalization of samples was performed by dividing the number of copies of both *E-cadherin* and *α-SMA* by the number of copies of *18S* and *GAPDH*. PCR reactions for *E-cadherin* and *α-SMA* cDNA quantification were performed using standard cDNA dilution curves. Quantitative data were analyzed statistically using a Student's *t*-test and expressed as means ± SEM. A value of $P < 0.05$ was considered significant.

Results

MMPIs Suppress Anterior Subcapsular Cataract Formation

In the following experiments, the broad spectrum MMP inhibitor GM6001 was used in the rat subcapsular cataract model to determine whether it could effectively suppress TGFβ-induced subcapsular cataract formation. To perform these experiments, excised rat lenses were treated with exogenous TGFβ2 for a period of 6 days. The TGFβ-treated lenses exhibited multiple, distinct opacities on the anterior surface of the lens, as previously described[27,34] (Figure 4B), whereas the untreated control lenses remained transparent and devoid of opacities (Figure 4A). In comparison with lenses treated with TGFβ alone, lenses co-cultured with TGFβ and GM6001 (25 μmol/L) for the 6-day period did not exhibit visible subcapsular cataracts (Figure 4C) but resembled the control lenses. Histological cross-sections of the lenses treated with TGFβ revealed the presence of numerous plaques consisting of a multilayering of cells beneath the lens capsule (Figure 4F) in contrast to the simple cuboidal monolayer of epithelial cells observed in the control lens (Figure 4E). Strong immunoreactivity of α-SMA was observed in a proportion of the cells of the subcapsular plaques in the TGFβ-treated lenses (Figure 4F). In comparison, the lenses co-cultured with TGFβ and GM6001 (25 μmol/L) did not exhibit multilayering of the lens epi-

thelium and no α-SMA immunoreactivity was observed under the same immunolocalization conditions as outlined above (Figure 4G).

In parallel with the lenses above, lenses were co-treated with TGFβ and the specific MMP2/9 inhibitor at concentrations of 10 or 25 μmol/L for 6 days. Similar to the findings for GM6001, these studies revealed that co-treatment with the MMP2/9 inhibitor suppressed the appearance of ASC plaques and α-SMA expression. Lenses co-treated with TGFβ and the MMP-2/9-specific inhibitor (10 μmol/L) exhibited slight multilayering of the epithelium (two layers observed) in some regions of the lens with very faint α-SMA immunoreactivity (data not shown). However, lenses co-treated with TGFβ and the MMP-2/9 inhibitor (25 μmol/L) resembled control lenses and did not exhibit α-SMA immunoreactivity (Figure 4, D and H). Note that lenses treated with each of the MMPIs, in the absence of TGFβ, resembled the control lenses (not shown).

A laser scanning system (ScanTox) was next used to determine quantitative differences in the optical quality of the lenses from three different treatment groups (control, TGFβ, and TGFβ plus 25 μmol/L GM6001) at three time points: days 2, 4, and 6. Analyses of BVD errors showed that there was both a treatment and temporal effect of TGFβ on the cultured rat lens (Figure 2). Although the BVD errors for the control lenses did not change significantly from day 0 to 6, treatment with either 1 or 2 ng/ml of TGFβ caused a significant increase in BVD error by day 6 of the time course. In contrast, the groups of lenses co-treated with TGFβ (at 1 and 2 ng/ml) and GM6001 (25 μmol/L) did not exhibit a significant change in BVD error over the 6-day period. Statistical differences between the treatment groups at each time point were also determined. At days 0, 2, and 4, no significant difference in BVD error was observed between all groups (Figure 2). However, at day 6, both of the TGFβ-treated groups of lenses (1 and 2 ng/ml) exhibited a significantly larger BVD error versus the control group and the GM6001 co-treated lenses (Figure 2). Lenses cultured with the inhibitor alone did not exhibit changes in BVD error relative to untreated lenses (not shown).

A dose-dependent effect of the broad spectrum inhibitor GM6001 in preventing the TGFβ-induced cataracts was also observed. Similar to earlier experiments, treatment with TGFβ for 6 days resulted in BVD errors that were statistically greater than control lenses. Similarly, those lenses co-treated with TGFβ and GM6001 (10 or 15 μmol/L) significantly differed from controls and exhibited cataracts. In contrast, lenses co-treated with higher concentrations of GM6001, such as 20 and 25 μmol/L, exhibited BVD errors that were very similar to the controls, further demonstrating the dramatic suppression in cataract formation (Figure 3). Treatment with an analog of GM6001, with no MMP inhibitory activity (negative control), exhibited a similar BVD error to the TGFβ-treated lens, indicating that the effect of GM6001 on maintaining lens optical quality was related to its MMP inhibitory activity.

TGFβ-Induced ASC Formation Is Accompanied by Enhanced Secretion of MMP-2 and MMP-9

To examine the timing and level of induction of MMPs in the rat lens after TGFβ treatment and subcapsular cataract formation, zymography was performed on conditioned media of lenses taken at the 6-day time point from the following treatment groups: control, TGFβ (2 ng/ml), TGFβ (2 ng/ml) plus GM6001 (25 μmol/L), and TGFβ (2 ng/ml) plus the MMP-2/9-specific inhibitor (25 μmol/L). Conditioned media from all treatment groups exhibited distinct bands on gelatin gels, indicating the presence of MMPs with gelatinolytic and/or collagenolytic activity (Figure 5A). Conditioned media from control lenses exhibited expression of a 92-kd band, corresponding to the proform of MMP-9.[11] In comparison with control lens media, media from lenses treated with TGFβ exhibited additional bands of 62, 65, and 72 kd, corresponding to the active and proforms of MMP-2;[11] MMP-9 levels were also increased. Media obtained from lenses co-treated with TGFβ and either GM6001 or MMP-2/9 inhibitor for 6 days exhibited reduced levels of all gelatinolytic bands relative to that of TGFβ-treated lenses (Figure 5A).

Confirmation and quantification of MMP-2 and MMP-9 from all treatment groups at each time period was performed using Western blot analysis. Blots developed with an MMP-2-specific antibody revealed the presence of latent and active species of MMP-2 in conditioned media from lenses treated with TGFβ, whereas the control lenses at all time points did not exhibit detectable levels of MMP-2 protein (Figure 5B). In comparison, conditioned media from the lenses co-cultured with TGFβ plus GM6001 or TGFβ plus the MMP-2/9 inhibitor showed undetectable levels of MMP-2 similar to control lenses (Figure 5B). Blots probed with the MMP-9-specific antibody revealed a band at 92 kd, corresponding to the proform of MMP-9 (Figure 5B). Similar to the zymography results, constitutive MMP-9 protein expression was evident in the conditioned media of control lenses. In comparison, media from lenses treated with TGFβ exhibited significantly higher levels of MMP-9 after 2, 4, and 6 days of treatment (Figure 5, B and C). Co-treatment with TGFβ and GM6001 revealed significant attenuation of MMP-9 in conditioned media relative to TGFβ treatment alone at all three time points, whereas conditioned media from co-treatment of TGFβ and the specific MMP-2/9 inhibitor showed a significant attenuation of MMP-9 at days 2 and 4 only (Figure 5, B and C). Note that lenses treated with either MMPI alone exhibited levels of MMP-9 and MMP-2 similar to that of control lenses (data not shown).

MMPIs Attenuate TGFβ-Induced E-Cadherin Shedding in the Rat Lens

Because previous studies had shown the ability of MMPs to induce E-cadherin shedding,[22–24] the following experiments were designed to determine whether

TGFβ treatment of the rat lens results in an induction of E-cadherin shedding and whether this can be modulated by the MMPIs. To accomplish this, previously concentrated conditioned media were obtained from the 2-, 4-, and 6-day treatment groups outlined earlier and subjected to Western blot analysis using an antibody specific for the extracellular domain of E-cadherin, which has been used in previous studies to detect the presence of soluble E-cadherin fragments. An E-cadherin fragment of approximately 72 kd was detected in the conditioned media from lenses treated with TGFβ for 6 days and was not observed in media from untreated lenses (Figure 6A). Media from lenses treated with TGFβ for shorter time periods (days 2 and 4) did not exhibit detectable levels of the fragment (not shown). Importantly, a significant twofold reduction in the levels of this fragment was detected in media from lenses co-treated with TGFβ and the broad spectrum inhibitor GM6001, whereas levels were undetectable in media from lenses co-treated with TGFβ and the MMP-2/9 inhibitor (Figure 6A). Lenses treated with either of the MMPIs alone also exhibited undetectable levels of the E-cadherin fragment (data not shown). Thus, co-culture of lenses with either of the MMPIs tested resulted in attenuated levels of TGFβ-induced E-cadherin fragment.

MMP inhibitors have been shown to augment cell-cell adhesion and specifically increase expression of cadherins.[23] We therefore examined the levels of *E-cadherin* mRNA using RT-QPCR. After 6 days of culture, we examined epithelial cells or plaque cells of lenses from the following treatment groups: control lenses, lenses treated with TGFβ (2 ng/ml), TGFβ (2 ng/ml) plus GM6001 (25 μmol/L), or GM6001 (25 μmol/L) alone. For these experiments, cryostat sections of lenses were subjected to LCM to specifically isolate the cells in the plaques. RT-QPCR findings revealed that whereas *E-cadherin* mRNA was detected in the normal lens epithelium (Figure 6B), its expression, relative to that of *GAPDH*, was suppressed nearly fourfold in the plaque tissue of TGFβ-treated lenses. In comparison, *E-cadherin* levels in cells from the epithelial region of lenses co-treated with TGFβ and GM6001 (25 μmol/L) were significantly higher (6.9-fold) than those treated with TGFβ alone and significantly greater than that of controls. Treatment with 25 μmol/L GM6001 alone also produced a significant increase in *E-cadherin* when compared with control lenses ($P < 0.01$) (2.5-fold). When these experiments were performed using the *18S* housekeeping gene, data exhibited the same trends seen with *GAPDH* (not shown). Thus, treatment of lenses with GM6001 prevented the attenuation of *E-cadherin* mRNA induced by TGFβ and

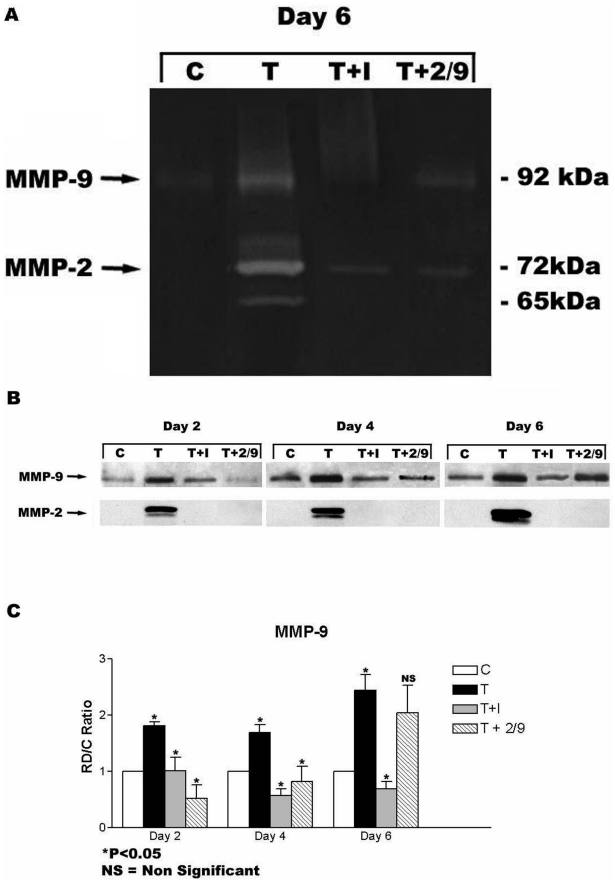
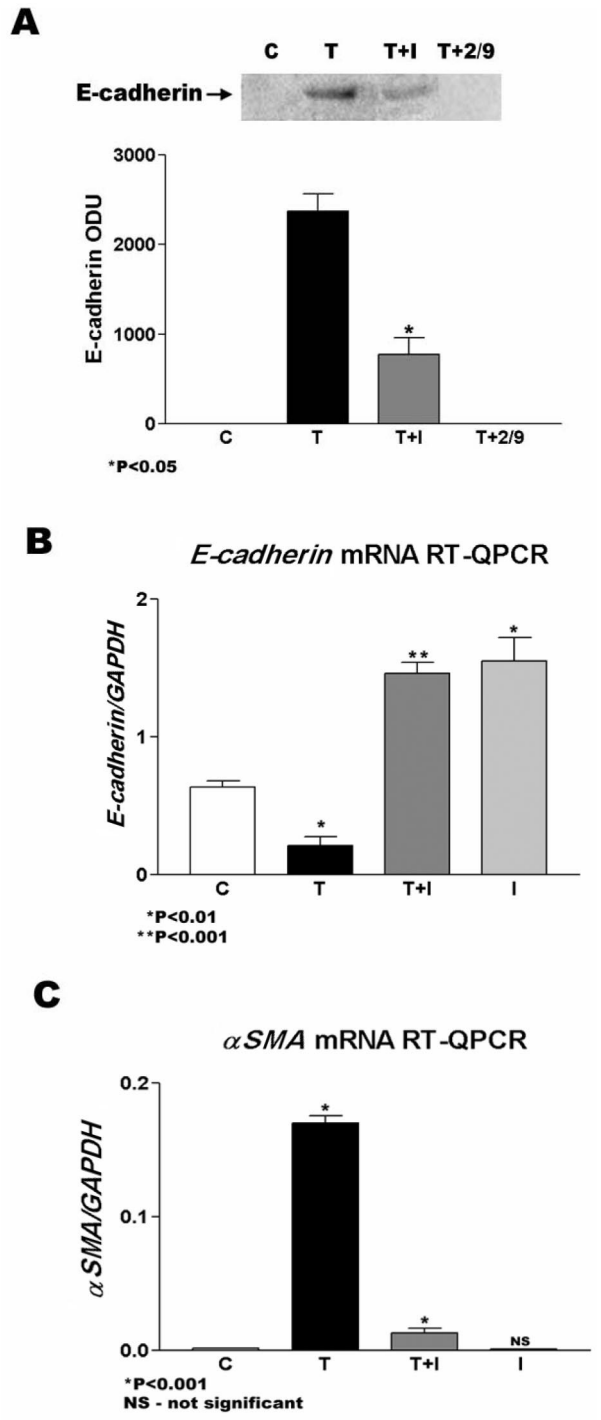


Figure 5. Effect of TGFβ and MMPIs on MMP protein levels. **A:** Gelatin zymography of conditioned media from cultured lenses after 6 days of treatment. MMP-9 (92 kd) was detected in media from all of the untreated (C) lenses at day 6 of the culture period. After treatment with TGFβ (2 ng/ml) (T), up-regulated levels of MMP-9 were observed relative to controls. Conditioned media obtained from lenses treated with TGFβ (2 ng/ml) and GM6001 (25 μmol/L) (T+I) exhibited attenuated bands and resembled levels secreted by control lenses. However, conditioned media obtained from lenses co-cultured with TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (T+2/9) exhibited up-regulated levels of MMP-9, relative to controls. Gelatinolytic bands for proMMP-2 (72 kd) and active MMP-2 (65 kd) were observed in conditioned media from the TGFβ-treated (2 ng/ml) (T) lenses but not in control (C) lenses. Media from TGFβ (2 ng/ml) and GM6001 (25 μmol/L) (T+I) and lenses co-cultured with TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (T+2/9) exhibited negligible activity for MMP-2. **B:** Representative Western blot. Conditioned media obtained from control lenses (C) and lenses treated with TGFβ (2 ng/ml) (T), TGFβ (2 ng/ml) and GM6001 (25 μmol/L) (T+I), and TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (T+2/9) at 2, 4, and 6 days were examined by Western blot analysis to confirm the identity of MMP-2 and MMP-9. A representative blot demonstrates that MMP-9 was constitutively expressed in all of the control lenses (C) over 2, 4, and 6 days. An up-regulation in MMP-9 protein was observed in media from lenses treated with TGFβ (2 ng/ml) (T) at day-2, -4, and -6 time points compared with control lenses (C), whereas the lenses co-cultured with TGFβ and GM6001 (25 μmol/L) (T+I) exhibited levels similar to the controls at all three time points. Lenses co-cultured with TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (T+2/9) exhibited an up-regulation in MMP-9 protein at day 6 compared with control lenses (C), whereas at day-2 and -4 time points, the lenses exhibited levels similar to the controls. At all three time points, MMP-2 protein was only detected in media from lenses treated with TGFβ (2 ng/ml) (T), whereas the other treatment groups did not exhibit detectable levels. **C:** Densitometric analysis of MMP-9 protein levels in conditioned media. The Western blot data for MMP-9 from three separate experiments were analyzed by densitometry. Values are expressed as the relative density versus control ratio (RD/C) ± SEM of three blots. Note a significant up-regulation ($*P < 0.05$) of MMP-9 in the conditioned media of TGFβ (2 ng/ml) (T)-treated lenses compared with control. A significant reduction ($*P < 0.05$) was observed in the expression of MMP-9 in conditioned media of lenses co-treated with TGFβ (2 ng/ml) and GM6001 (25 μmol/L) (T+I) compared with TGFβ (2 ng/ml) (T). In the conditioned media of lenses co-cultured with TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (T+2/9), a significant reduction ($*P < 0.05$) was observed in the expression of MMP-9 at day-2 and -4 time points compared with TGFβ (2 ng/ml) (T), whereas there was no significant reduction at day 6 compared with TGFβ (2 ng/ml) (T).

further increased the constitutive levels of *E-cadherin* mRNA.

For comparison, we also examined the levels of α-*SMA* mRNA in the same tissues used to perform the *E-cadherin* studies (Figure 6C). These experiments revealed little to no expression of α-*SMA* mRNA in the cells obtained from the epithelial region of control lenses whereas its expression, relative to that of *GAPDH*, was significantly induced in the plaque tissue of TGFβ-treated lenses. This corresponds with our earlier experiments, which showed increased α-SMA protein immunoreactivity in the subcapsular plaques (Figure 4F). α-*SMA* mRNA levels in the LECs co-treated with the MMPI were substantially reduced compared with the plaque cells of TGFβ-treated lenses, demonstrating that co-culture with the MMPI resulted in suppression in induction of α-*SMA* by TGFβ. Addition of GM6001 (25 μmol/L) alone to lenses did not alter α-*SMA* mRNA levels when compared with those of control lenses (Figure 6C).



Discussion

Increasing evidence regarding the importance of MMPs in development and pathology has accumulated over the last 10 years. In the eye, MMPs have been shown to contribute to a number of ocular diseases and disorders including retinal disease, glaucoma, corneal ulcers and corneal postoperative haze.[12] More recently, induction of MMP expression has been correlated with the formation of cataracts.[16,18,19] However, the role of MMPs and the mechanism by which they contribute to cataractogenesis are not well understood. In the current study, we used the previously established *ex vivo* rat lens subcapsular cataract model to directly test the involvement of MMPs in the initiation and progression of TGFβ-induced ASC. We demonstrated that co-treatment with TGFβ and two different MMPIs, the broad spectrum inhibitor GM6001 and the MMP-2/9-specific inhibitor, resulted in suppression of TGFβ-induced ASC formation, including the appearance of α-SMA-expressing cells indicative

Figure 6. Effect of TGFβ and GM6001 on α-SMA and E-cadherin mRNA and shedding. **A:** Representative Western blot and densitometric analysis of E-cadherin protein released into conditioned media. The Western blot revealed the presence of a 72-kd E-cadherin fragment in the conditioned media from lenses treated with TGFβ (2 ng/ml) (T) that was not detected in media from control lenses (C) and lenses co-treated with TGFβ (2 ng/ml) and MMPI2/9 (25 μmol/L) (T+2/9). Levels of the E-cadherin fragment were suppressed in media from lenses co-cultured with TGFβ and GM6001 (25 μmol/L) (T+I), relative to that from TGFβ-treated lenses. Values are expressed as optical density units (ODU) $\pm$ SEM of three blots. Note the presence of E-cadherin fragment in conditioned media of TGFβ-treated (2 ng/ml) (T) lenses compared with control (C). A significant ($^*P < 0.05$) reduction in the levels of the E-cadherin fragment was observed in media from lenses co-treated with TGFβ (2 ng/ml) and GM6001 (25 μmol/L) compared with TGFβ (T). **B:** *E-cadherin* mRNA expression using RT-QPCR. *E-cadherin* mRNA levels, relative to *GAPDH*, were also measured for the lenses treated in **A** using RT-QPCR, and the values are given as $\pm$SEM of three separate experiments. All comparisons were two tailed. The RT-QPCR results revealed that cataractous plaques derived from lenses treated with TGFβ (2 ng/ml) (T) for 6 days exhibited a significant suppression ($^*P < 0.01$) in *E-cadherin* mRNA levels compared with epithelial cells from control lenses (C). Lenses co-cultured with TGFβ and GM6001 (25 μmol/L) (T+I) exhibited significantly higher levels ($^{**}P < 0.001$) of *E-cadherin* compared with those treated with TGFβ (2 ng/ml) (T) (nearly sevenfold). The GM6001 (25 μmol/L) alone group (I) exhibited significantly elevated levels of *E-cadherin* relative to control (C) lenses ($^*P < 0.01$). **C:** α-*SMA* mRNA expression using RT-QPCR. The α-SMA mRNA levels were measured relative to *GAPDH*, and the values are given as $\pm$SEM. All comparisons were two tailed. The RT-QPCR results revealed that the control lens epithelium (C) expressed minimal levels of α-*SMA* mRNA. The cells isolated from the cataractous plaques of the TGFβ-treated (2 ng/ml) (T) lenses expressed significantly higher levels ($^*P < 0.001$) of α-*SMA* mRNA compared with epithelial cells from control lenses (C). Lens epithelium from lenses co-cultured with TGFβ (2 ng/ml) and GM6001 (25 μmol/L) (T+I) exhibited significantly less ($^*P < 0.001$) α-*SMA* mRNA compared with those treated with TGFβ (2 ng/ml) (T). Lenses treated with GM6001 alone (25 μmol/L) did not exhibit statistically significant elevated levels of α-SMA when compared with control lenses (C).

of the EMT of LECs. Further evidence is provided to show that treatment of the rat lens with TGFβ resulted in appearance of proteolytic fragments of the cell-cell adhesion molecule E-cadherin, an event that was attenuated by co-treatment with either of the two MMPIs tested. Together, these data suggest that the suppression of TGFβ-mediated E-cadherin shedding and degradation is a possible mechanism by which MMPIs reduce the appearance of ASC plaques.

Earlier work has shown that *MMP-2* expression is induced in the rat lens after treatment with TGFβ.[16] Furthermore, overexpression of *MMP-2* via stable transfection results in conversion of cultured human LECs into α-SMA-expressing cells.[16] Similar to these findings, we also report induced secretion of MMP-2 in the rat lens after treatment with TGFβ, accompanied by enhanced secretion of MMP-9. A recent study revealed that *MMP-9* and *MMP-2* mRNA are expressed in the normal rat lens epithelium.[35] This suggests that constitutive mRNA expression of these MMPs does not result in the EMT of LECs and cataract formation. However, induced levels of *MMP-2* and/or *MMP-9*, above those of constitutive expression, and accompanying secretion of MMPs could result in EMT in the lens. Induced levels of MMP-2 and membrane-type 1-MMP have been correlated with EMT in the embryonic heart, and overexpression of MMP-3 has been shown to cause conversion of mammary epithelial cells into mesenchymal cells.[21,36] The fact that co-treatment with GM6001 and the specific MMP2/9 inhibitor resulted in suppression of TGFβ-induced α-SMA expression in the rat lens, further corroborates these earlier findings and suggests that MMPs are important for mediating the EMT of LECs. We further show that treatment with MMPIs led to a substantial reduction in subsequent plaque formation in the *ex vivo* lens and maintenance of lens optical quality. Because MMP-2 and MMP-9 were induced after TGFβ treatment and the MMPIs used have inhibitory activity against both, it cannot be discerned whether one or both of these MMPs participate in ASC formation. Future studies that target the individual expression of MMP-2 or MMP-9, such as through gene knockdown experiments or the use of specific *MMP-2* and *MMP-9* knockout mouse models, will help to further determine whether one or both of these MMPs is critical for ASC formation.

MMPs are principally known for their role in extracellular matrix (ECM) remodeling.[11] However, additional roles for MMPs have emerged, including their ability to regulate cell migration, invasion, and EMT.[21,36] It has been suggested that MMPs may contribute to EMT by participating in the separation of epithelial cells from their basement membrane,[37,38] thereby promoting their migration during cellular transformation.[7,37,38] However, more recent findings suggest that MMPs participate in the initial activation stages of EMT through dissociation of the E-cadherin/β-catenin complex.[22–24] Proteolytic cleavage of the N-terminal extracellular domain of E-cadherin by MMPs, referred to as "E-cadherin shedding," results in the formation of an E-cadherin extracellular domain fragment with reported sizes ranging from 50 to 84 kd, compared with the intact 120-kd protein.[39] Here, we report the appearance of a 72-kd E-cadherin fragment in the conditioned media of lenses treated with TGFβ that was not detected in the media from untreated lenses. To the best of our knowledge, this is the first report of E-cadherin shedding in a cataract model. The appearance of the E-cadherin fragment in the TGFβ-treated rat lenses was also shown to coincide with enhanced levels of secreted MMP-2 and MMP-9, MMPs that have been implicated in E-cadherin shedding and degradation in other systems.[24,40] We further demonstrated that the TGFβ-induced levels of the E-cadherin fragment were attenuated by co-treatment with either MMPI, broad spectrum GM6001, or MMP-2/9 inhibitor, suggesting that TGFβ-induced fragmentation of E-cadherin is mediated by MMP activity. Interestingly, the ability of GM6001 to suppress E-cadherin shedding has been shown in nitric oxide-treated murine colonic epithelial cells, and this led to further stabilization of E-cadherin junctions.[22,24]

Along with E-cadherin shedding, we also reported a significant decrease in the *E-cadherin* mRNA levels in the subcapsular plaque tissue of TGFβ-treated lenses. Decreased *E-cadherin* expression has also been reported after dexamethasone-induced cataract formation in the cultured rat lens; however, evidence of E-cadherin shedding in this cataract model was not investigated.[41] Cadherins are known to act as cell signaling receptors by controlling the localization of β-catenin. Proteolytic shedding of E-cadherin causes dissociation of β-catenin from membrane-bound E-cadherin, resulting in increases in the levels of free β-catenin in the cytoplasm.[24] β-Catenin can then localize to the nucleus where it associates with the T-cell factor/lymphoid enhancer factor(s) to activate or repress target gene expression.[22] Thus, the changes in *E-cadherin* and *α-SMA* mRNA levels that we observed may be associated with changes in β-catenin localization. The levels of *E-cadherin* mRNA in lenses co-treated with TGFβ and GM6001 were found to be well above that of control lenses, and treatment of lenses with GM6001 alone also resulted in a substantial elevation in the constitutive amount of *E-cadherin* mRNA. These findings suggest that *E-cadherin* mRNA expression was either stimulated or stabilized by GM6001.

The findings of the current study also revealed that treatment with the broad spectrum inhibitor GM6001 suppressed TGFβ-induced levels of both MMP-2- and MMP-9-secreted protein, whereas the MMP-2/9 inhibitor specifically blocked MMP-2 induced levels. The primary function of both MMPIs is to inhibit MMP enzymatic activity. However, MMPIs such as GM6001 have been shown to have an inhibitory effect on MMP expression in other systems, yet this has remained unexplained.[42,43] Previous work has shown that the myofibroblast-like cells in the subcapuslar plaques of rat lenses treated with TGFβ exhibited immunoreactivity for both α-SMA and MMP-2.[16] Thus, the absence of MMP-2-secreted protein in the media from lenses co-treated with either of the two MMPIs may simply be due to the fact that both MMPIs suppress the appearance of myofibroblasts, the cell type that expresses MMP-2.

In summary, the findings of the current study have shown that treatment with the broad spectrum MMP inhibitor GM6001 and the specific MMP2/9 inhibitor significantly suppressed the formation of TGFβ-induced ASC plaques in the rat lens. This suppression was associated with decreased α-SMA expression and a significant decrease in the 72-kd soluble E-cadherin fragment, indicative of E-cadherin shedding. Together, these data suggest the novel finding that MMPIs suppress the EMT of LECs in ASC formation through inhibition in MMP-mediated disruption of E-cadherin. Based on these findings, two possible therapeutic strategies for the prevention of ASC formation can be proposed: inhibition of MMP activity and/or attenuation of aberrant E-cadherin shedding.

Acknowledgments

We thank Giuseppe Pontoriero and Paula Deschamps for their technical assistance.

References

1. McCarty CA, Taylor HR: Recent developments in vision research: light damage in cataract. Invest Ophthalmol Vis Sci 1996, 37:1720–1723
2. Griep AE, Zhang P: Lens cell proliferation. Development of the Ocular Lens. Edited by FJ Lovicu, ML Robinson. New York, Cambridge University Press, 2004, pp 191–213
3. Lang RA, McAvoy JW: Growth factors in lens development. Development of the Ocular Lens. Edited by FJ Lovicu, ML Robinson. New York, Cambridge University Press, 2004, pp 261–289
4. Sasaki K, Kojima M, Nakaizumi H, Kitagawa K, Yamada Y, Ishizaki H: Early lens changes seen in patients with atopic dermatitis applying image analysis processing of Scheimpflug and specular microscopic images. Ophthalmologica 1998, 212:88–94
5. Font R, Brownstein S: A light and electron microscopic study of anterior subcapsular cataracts. Am J Ophthalmol 1974, 78:972–984
6. Novotny GE, Pau H: Myofibroblast-like cells in human anterior capsular cataract. Virchows Arch A Pathol Anat Histopathol 1984, 404:393–401
7. Hay ED: An overview of epithelio-mesenchymal transformation. Acta Anat (Basel) 1995, 154:8–20
8. Lovicu FJ, Schulz MW, Hales AM, Vincent LN, Overbeek PA, Chamberlain CG, McAvoy JW: TGFbeta induces morphological and molecular changes similar to human anterior subcapsular cataract. Br J Ophthalmol 2002, 86:220–226
9. Kappelhof JP, Vrensen GF: The pathology of after-cataract: a minireview. Acta Ophthalmol Suppl 1992, 205:13–24
10. Marcantonio JM, Syam PP, Liu CS, Duncan G: Epithelial transdifferentiation and cataract in the human lens. Exp Eye Res 2003, 77:339–346
11. Alexander CM, Werb Z: Proteinases and extracellular matrix remodeling. Curr Opin Cell Biol 1989, 1:974–982
12. Sivak JM, Fini ME: MMPs in the eye: emerging roles for matrix metalloproteinases in ocular physiology. Prog Retin Eye Res 2002, 21:1–14
13. Smine A, Plantner JJ: Membrane type-1 matrix metalloproteinase in human ocular tissues. Curr Eye Res 1997, 16:925–929
14. Reponen P, Sahlberg C, Munaut C, Thesleff I, Tryggvason K: High expression of 92-kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. J Cell Biol 1994, 124:1091–1102
15. Mohan R, Rinehart WB, Bargagna-Mohan P, Fini ME: Gelatinase B/lacZ transgenic mice, a model for mapping gelatinase B expression during developmental and injury-related tissue remodeling. J Biol Chem 1998, 273:25903–25914
16. Seomun Y, Kim J, Lee EH, Joo CK: Overexpression of matrix metalloproteinase-2 mediates phenotypic transformation of lens epithelial cells. Biochem J 2001, 358:41–48
17. Richiert DM, Ireland ME: Matrix metalloproteinase secretion is stimulated by TGF-beta in cultured lens epithelial cells. Curr Eye Res 1999, 19:269–275
18. Wormstone IM, Tamiya S, Anderson I, Duncan G: TGF-beta2-induced matrix modification and cell transdifferentiation in the human lens capsular bag. Invest Ophthalmol Vis Sci 2002, 43:2301–2308
19. Tamiya S, Wormstone IM, Marcantonio JM, Gavrilovic J, Duncan G: Induction of matrix metalloproteinases 2 and 9 following stress to the lens. Exp Eye Res 2000, 71:591–597
20. Kawashima Y, Saika S, Miyamoto T, Yamanaka O, Okada Y, Tanaka S, Ohnishi Y: Matrix metalloproteinases and tissue inhibitors of metalloproteinases of fibrous humans lens capsules with intraocular lenses. Curr Eye Res 2000, 21:962–967
21. Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ, Werb Z: The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 1999, 98:137–146
22. George SJ, Dwivedi A: MMPs, cadherins, and cell proliferation. Trends Cardiovasc Med 2004, 14:100–105
23. Ho AT, Voura EB, Soloway PD, Watson KL, Khokha R: MMP inhibitors augment fibroblast adhesion through stabilization of focal adhesion contacts and up-regulation of cadherin function. J Biol Chem 2001, 276:40215–40224
24. Mei JM, Borchert GL, Donald SP, Phang JM: Matrix metalloproteinase(s) mediate(s) NO-induced dissociation of beta-catenin from membrane bound E-cadherin and formation of nuclear beta-catenin/LEF-1 complex. Carcinogenesis 2002, 23:2119–2122
25. Noe V, Fingleton B, Jacobs K, Crawford HC, Vermeulen S, Steelant W, Bruyneel E, Matrisian LM, Mareel M: Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. J Cell Sci 2001, 114:111–118
26. Hirai Y, Migita K, Honda S, Ueki Y, Yamasaki S, Urayama S, Kamachi M, Kawakami A, Ida H, Kita M, Fukuda T, Shibatomi K, Kawabe Y, Aoyagi T, Eguchi K: Effects of nitric oxide on matrix metalloproteinase-2 production by rheumatoid synovial cells. Life Sci 2001, 68:913–920
27. Hales AM, Chamberlain CG, McAvoy JW: Cataract induction in lenses cultured with transforming growth factor-beta. Invest Ophthalmol Vis Sci 1995, 36:1709–1713
28. Priolo S, Sivak JG, Kuszak JR, Irving EL: Effects of experimentally induced ametropia on the morphology and optical quality of the avian crystalline lens. Invest Ophthalmol Vis Sci 2000, 41:3516–3522
29. Bantseev V, McCanna D, Banh A, Wong WW, Moran KL, Dixon DG, Trevithick JR, Sivak JG: Mechanisms of ocular toxicity using the in vitro bovine lens and sodium dodecyl sulfate as a chemical model. Toxicol Sci 2003, 73:98–107
30. Oriowo OM, Cullen AP, Chou BR, Sivak JG: Action spectrum and recovery for in vitro UV-induced cataract using whole lenses. Invest Ophthalmol Vis Sci 2001, 42:2596–2602
31. Heussen C, Dowdle EB: Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. Anal Biochem 1980, 102:196–202
32. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA: Laser capture microdissection. Science 1996, 274:998–1001
33. Bonner RF, Emmert-Buck M, Cole K, Pohida T, Chuaqui R, Goldstein S, Liotta LA: Laser capture microdissection: molecular analysis of tissue. Science 1997, 278:1481–1483
34. Lovicu FJ, Steven P, Saika S, McAvoy JW: Aberrant lens fiber differentiation in anterior subcapsular cataract formation: a process dependent on reduced levels of Pax6. Invest Ophthalmol Vis Sci 2004, 45:1946–1953
35. John M, Jaworski C, Chen Z, Subramanian S, Ma W, Sun F, Li D, Spector A, Carper D: Matrix metalloproteinases are down-regulated in rat lenses exposed to oxidative stress. Exp Eye Res 2004, 79:839–846
36. Song W, Jackson K, McGuire PG: Degradation of type IV collagen by matrix metalloproteinases is an important step in the epithelial-mesenchymal transformation of the endocardial cushions. Dev Biol 2000, 227:606–617
37. Boyer AS, Erickson CP, Runyan RB: Epithelial-mesenchymal transformation in the embryonic heart is mediated through distinct pertus-

sis toxin-sensitive and TGFbeta signal transduction mechanisms. Dev Dyn 1999, 214:81–91
38. Brown CB, Boyer AS, Runyan RB, Barnett JV: Requirement of type III TGF-beta receptor for endocardial cell transformation in the heart. Science 1999, 283:2080–2082
39. Steinhusen U, Weiske J, Badock V, Tauber R, Bommert K, Huber O: Cleavage and shedding of E-cadherin after induction of apoptosis. J Biol Chem 2001, 276:4972–4980
40. Nawrocki-Raby B, Gilles C, Polette M, Bruyneel E, Laronze JY, Bonnet N, Foidart JM, Mareel M, Birembaut P: Upregulation of MMPs by soluble E-cadherin in human lung tumor cells. Int J Cancer 2003, 105:790–795
41. Lyu J, Kim JA, Chung SK, Kim KS, Joo CK: Alteration of cadherin in dexamethasone-induced cataract organ-cultured rat lens. Invest Ophthalmol Vis Sci 2003, 44:2034–2040
42. Kerkvliet EH, Jansen ID, Schoenmaker TA, Docherty AJ, Beertsen W, Everts V: Low molecular weight inhibitors of matrix metalloproteinases can enhance the expression of matrix metalloproteinase-2 (gelatinase A) without inhibiting its activation. Cancer 2003, 97:1582–1588
43. Lei TC, Vieira WD, Hearing VJ: In vitro migration of melanoblasts requires matrix metalloproteinase-2: implications to vitiligo therapy by photochemotherapy. Pigment Cell Res 2002, 15:426–432